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Tony-*
Non-specific "Effect" Not Exposure

Dear Warren:

Thanks for the opportunity to comment on the Philip Morris population exposure study. We have a number of relevant suggestions, and would look forward to working with you to introduce the concepts and information to your client. I have organized suggestions as follows:

- Metaprobe™ biomarkers that could add a new dimension to the proposed study and are available for research implementation with established protocols
- More research-oriented biomarkers that have been tested in animals or in benchtop settings that could be accelerated in development for the proposed study
- Technical suggestions on the appropriateness or potential problems with selected biomarkers

We propose below four biomarkers that are directly relevant to understanding individual response to inhaled cigarette smoke. Established Metaprobe™ biomarkers include different capacity measures in: 1) liver function related to smoking history and metabolism of inhaled aryl hydrocarbons, 2) immune capacity and the ability to respond to free radical elevation and mitigate free radical-mediated damage, 3) vascular endothelial functional status, and 4) mitochondrial function and physiologic ATP capacity.

Liver Function

It is well known that aryl hydrocarbons as products of combustion result in up-regulation of the cytochrome P450 (CYP1A2) system. This liver enzyme sub-system is important in the metabolism of a number of widely used drug classes, including anti-depressants, proton pump inhibitors, and beta-blockers. MassTrace offers a liver function breath test based on labeled caffeine that can measure this up-regulation and compare the impact among subjects with different smoking habits and with different concomitant prescriptions.

This up-regulation is persistent for several days after exposure to aryl hydrocarbons, as with smoking. Thus, this test can be used to screen non-smokers in the control arm to assure that they are not 'closet smokers' or otherwise exposed to smoke in daily life.

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Immune Capacity

Cigarette smoking is associated with diminished immune capacity due to loss of cytoprotection mechanisms and oxidative stress. MassTrace has developed a breath test that measures the body's resting level of oxidant stress and immune system capacity to adapt to environmental insult. The breath test measures the body's ability to produce glutathione, the body's primary free radical scavenger. MassTrace has validated this test in healthy and diseased young and old populations, and when combined with a Tylenol challenge can allow comparisons across subjects in adaptive capacity as a proxy for the impact of chronic smoking on resting and adaptive capacity.

Mitochondrial Capacity

Cigarette smoking is associated with mutations in mitochondrial function. MassTrace has developed a breath test that measures this capacity using an index of ketoacid metabolism. If differences are observed in this basic assessment of the body's energy 'factories', blood samples can be used on a post hoc basis to look for actual evidence of mitochondrial mutations.

Vascular Epithelial Health

MassTrace offers measurements of nitric oxide production based on arginine and homologue metabolism. This test, unlike the three above, would be based on a plasma or urinary assay. Nitric oxide production changes have been associated with atherosclerosis, stroke and heart attack, inflammatory diseases of the lung, such as asthma, emphysema, and COPD, and gastrointestinal inflammation.

Other Metaprobes to Consider

The biomarkers above are ready for implementation in clinical trials with well-established protocols and experience in human subjects. The following biomarkers are directly relevant to a number of lung functions and lung injury. They could be accelerated in our development programs if they were of interest to Philip Morris scientists and any could be ready or human use in a maximum of six to twelve months.

Enzyme Target	Biomarker Application
Carboxypeptidase M	Macrophage differentiation
Caspase 1 (ICE)	Pro-inflammatory cytokine maturation/sepsis
Caspase 3	Apoptosis/cell repair
Caspase 8	Apoptosis (Fas mediated)
Cathepsin B-Enz & L	Apoptosis/cell repair, Tumor invasion
Cathepsin G & H	Phagosome/PMN capacity
Chymase	Inflammatory response (lung)
Leucocyte elastase	Inflammatory response (lung)
Macrophage elastase (MMP-12)	Inflammatory response (systemic)
Myeloblastin	Inflammatory response Factor activation (TNF, TGF)

Source: https://www.industrydocuments.ucsf.edu/docs/qqlx0001

Technical Suggestions

Acetonitrile – In addition to this biomarker, 3-ethenyl-pyridines should also be considered. They are most often used to measure smoke exposure in occupational environments and are another indicator of tobacco combustion.

Hb adducts of 3- and 4- aminobiphenyl – according to Benowitz, this indicator is of low sensitivity and specificity in blood so the information yield may be marginal.

Nicotine and nicotine metabolites – use of urine instead of plasma introduces potentially confounding variance based on population differences in renal clearance rates. Advances in GC/MS have made a plasma assay (using deuterated analytical standards in an isotope dilution protocol) more practical and likely more accurate.

Thromboxane B2 and Fibrinogen – These are not the most accepted markers for atherosclerosis and cardiovascular disease, respectively. Several national consensus groups have recommended biomarkers for these disease processes.

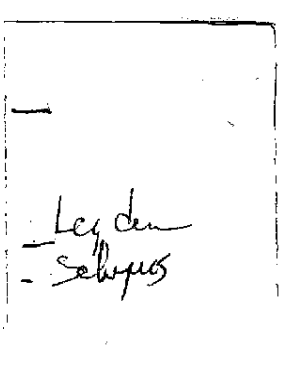
Isoprostanes – Not all are good biomarkers for atherosclerosis and lipid peroxidative metabolism. Might want to focus on a specific isoprostane, as well as breath isopentane and other volatile organics indicative of liver and lung metabolism. Analytically, this might be accomplished with a 'lab-on-a-chip' biosensor, which may be obtained from a number of suppliers using field effect transistor technology for real time monitoring.

Bronchial cells – Induced sputum is difficult to obtain consistently in all subjects. Might want to consider a nasal lavage technique, which also will be more robust, less subject to methodology differences, and validated in many studies on upper airway inflammation and smoking.

We have attached a preliminary bibliography supportive of the points made above. It may be appropriate to spend some time on a more comprehensive review of the literature. As described, there are newer techniques for the establishing the functional phenotype for smokers. It may be appropriate to review these in more detail prior to settling on test specifications that may not otherwise have as much prospective value for data mining. In addition, we could comment on the study design for epidemiological credibility and utility. As one example, urine collections would need to be more frequent than once daily to allow meaningful conclusions to be drawn about some urine-based biomarkers.

Please let Alfred or me know if we can assist you with clarification or additional materials.

Sincerely,



Peter B. Leone
President and CEO
cc: Alfred M. Ajami, Ph.D.

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